Docket No: 22740-2

PATENT

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Holly D. Kozlowski

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Sándor Sipka et al	Confirmation No.:	8175
Serial No.:	10/651,136	Group Art Unit:	1644
Filed:	August 28, 2003	Examiner:	Rooney, Nora Maureen

For: **Processes For Inhibiting Development of Allergic Disease**

DECLARATION UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Dr. Sándor Sipka declares that:

1. He is a co-inventor of and familiar with the present application Serial No. 10/651,136 filed on August 28, 2003, is familiar with the Official Action dated May 16, 2007, and the references cited therein, specifically, Malling et al, "Bacterial Vaccines: Anything but Placebo," *Allergy*, 55:214-218 (2000), Previte et al, "Detoxification of *Salmonella typhimurium* Lipopolysaccharide by Ionizing Radiation," *Journal of Bacteriology*, 93(5):1607-1614 (1967), Tarpley et al, "Radiation Sterilization," *Journal of Bacteriology*, 65(5):305-309 (1953), Schultz et al, "Radiation Degradation of Polymethacrylates. Dose Rate and Medium Effects," *Journal of Polymer Science: Part A*, 1:1651-1669 (1963), Matricardi et al, "Microbial Products in Allergy Prevention and Therapy", *Allergy*, 58:461-471 (2003), Gereda et al, "Relation Between House-dust Endotoxin Exposure, Type 1 T-cell Development, and Allergen Sensitization in Infants at High Risk of Asthma," *The Lancet*, 355:1680-1683 (2000), Oehling et al, "Bacterial

Immunotherapy of Childhood Bronchial Asthma," *Allergol. et Immunopathol.*, VIII:177-184 (1980) and Tulic et al, "Modification of the Inflammatory Response to Allergen Challenge After Exposure to Bacterial Lipopolysaccharide;" *Am. J. Resp. Cell Mol. Biol.*, 22:604-612 (2000).

2. He holds the position of Chief of the Regional Immunological Laboratory, Third Department of Internal Medicine, University of Debrecen, Research Center for Molecular Medicine, Medical and Health Science Center, Debrecen, Hungary and is knowlegable in the art of immunology.

3. The present invention is based on the discovery of a unique immune response elicited by irradiation-detoxified (IR) lipopolysaccharide (LPS) and the use of the IR-LPS in a method of decreasing development of allergic asthma by exposing a neonatal or immature mammal to the IR-LPS. To demonstrate the unexpected and surprising results of the present methods, the experiments described herein were conducted under his direction and control to compare the immune response elicited by IR-LPS with the immune response elicited by native LPS.

4. Human blood samples were obtained from volunteers and were heparinized using conventional techniques. To respective samples, 10 µg of either native LPS or IR-LPS was added and these samples, along with control samples to which neither native LPS nor IR-LPS was added, were then incubated for 4 hours at 37° C. Plasma cytokines (IL-1, TNF alpha, IFN gamma, IL-4 and IL-10) were then extracted and their concentration was determined by ELISA assays, while CD 69 expression was assayed by flow cytometry, all using conventional techniques.

5. The measured results were as follows:

Cytokine concentration and % CD69 expression	**Control, Unstimulated** Whole Blood Lymphocytes and Monocytes	**LPS Stimulated** Whole Blood Lymphocytes and Monocytes	**IR-LPS Stimulated** Whole Blood Lymphocytes and Monocytes

IL-1 beta pg/ml (average) n=3	9.1	172.3	98.2
TNF alpha (pg/ml)(average) n=3	19.3	1721.3	1596.2
IFN gamma (pg/ml)(average) n=3	13.7	42.2	11.0
IL-4 (pg/ml) (Atopic subject) n=1	< 0.4	7.8	2.0
IL-4 (pg/ml)(average) (Healthy subjects) n=2	< 0.4	< 0.4	< 0.4
IL-10 (pg/ml)(average) n=3	3.9	13.2	22.4
(%) CD69 (average) n=3	0.53	9.4	5.9

6. The results set forth in the above table demonstrate that IR-LPS stimulates the production of IL-10 significantly better than native LPS. This is significant because IL-10 is the key cytokine for the suppression of allergic response and inflammation. Further, IR-LPS elicits lesser IL-1 beta production, TNF alpha production, and IFN gamma production, indicating a positive but attenuated Th-1 response, and a lower % in CD-69 "activation markers" expressed by T lymphocytes. CD-69 is elevated in asthmatic subjects, therefore the lesser activation of this "activation marker" by IR-LPS indicates an attenuated asthmatic response (see, Yoshimura et al, "Activation markers of human basophils: CD69 expression is strongly and preferentially induced by IL-3," *J. Allergy Clin Immunol*, 109(5):817-823 (2002)). The IR-LPS also elicits a smaller increase in IL-4 production in atopic subjects, but not in normal subjects, indicating a reduced Th-2 response. Accordingly, as the high concentration and long-lasting native endotoxin exposure can result in wheezing and rash in infancy (J. Gillespie et al, *J Allergy Clin Immunol.*, 118:1265-70 (2006)), the use of IR-LPS diminishes these undesirable responses while still

stimulating the production of the Th-1 related cytokines, IL-1 beta, TNF alpha, and IFN gamma. This is a positive effect from the aspects of allergy prevention, with less side effects than would be produced with native LPS. Also, it is advantageous for allergic neighbors living in the IR-LPS-containing milieu because their IL-4 (Th-2 arm) is not stimulated as intensively as it would be if native LPS would be used.

7. The immune stimulating properties of IR-LPS are neither taught nor suggested by any of the prior art cited in the Official Action as noted above. Thus, none of this prior art, alone or in combination, suggests any benefit of using IR-LPS, particularly as compared with native LPS, in a method of decreasing development of allergic asthma. Accordingly, none of this prior art, alone or in combination, suggests a method of decreasing development of allergic asthma by exposing a neonatal or immature mammal to IR-LPS.

8. He further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

15/10/2007
Date

Sándor Sipka

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